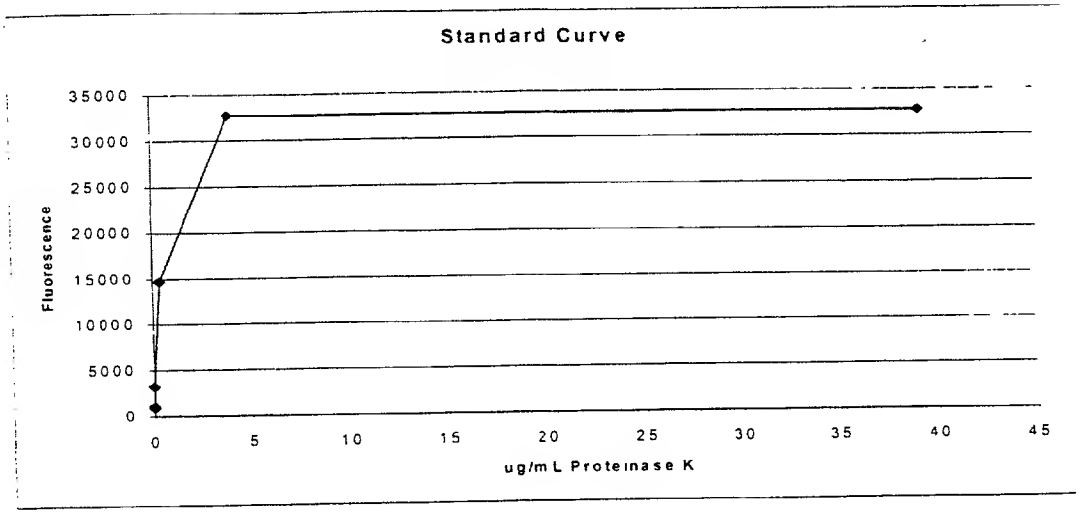

Standard Curve
Fluorescence
35000
30000
25000
20000
15000
10000
5000
0
0
5
10
15
20
25
30
35
40
45
ug/mL Proteinase K

Figure 1

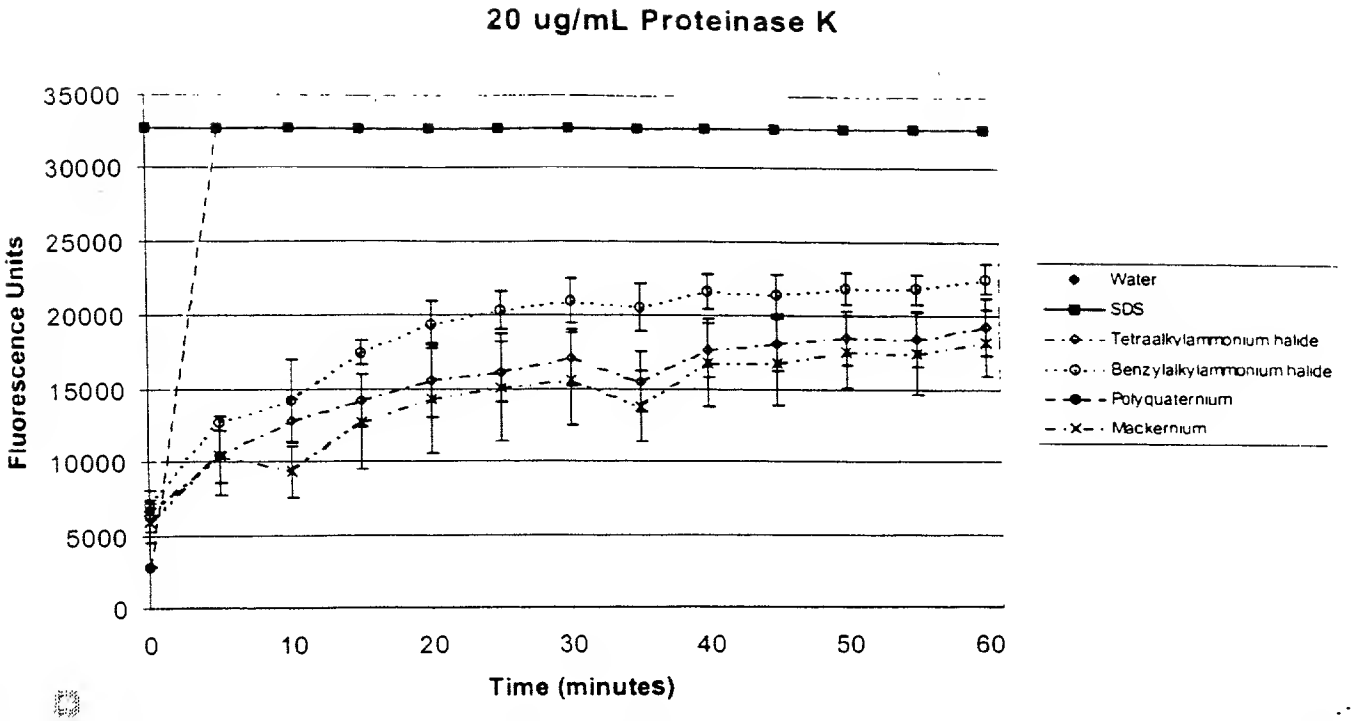


Figure 2A

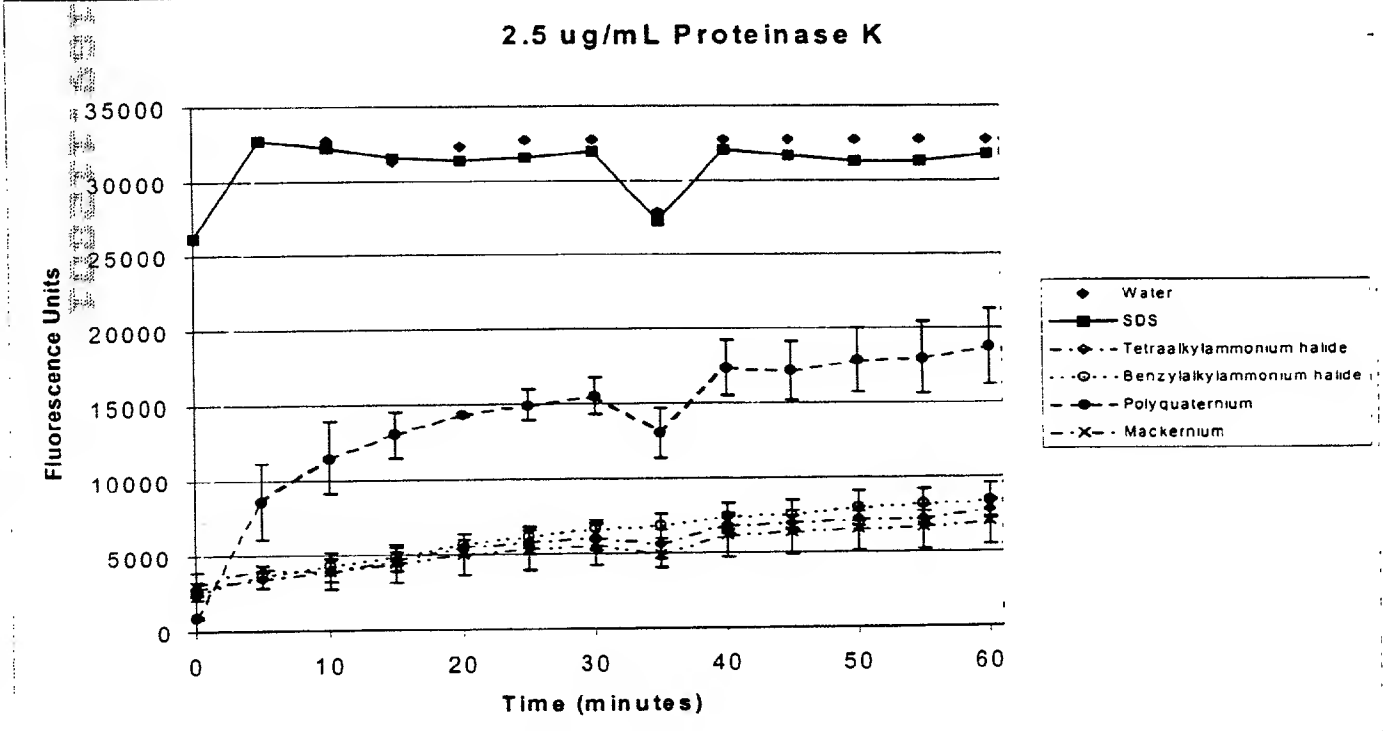


Figure 2B

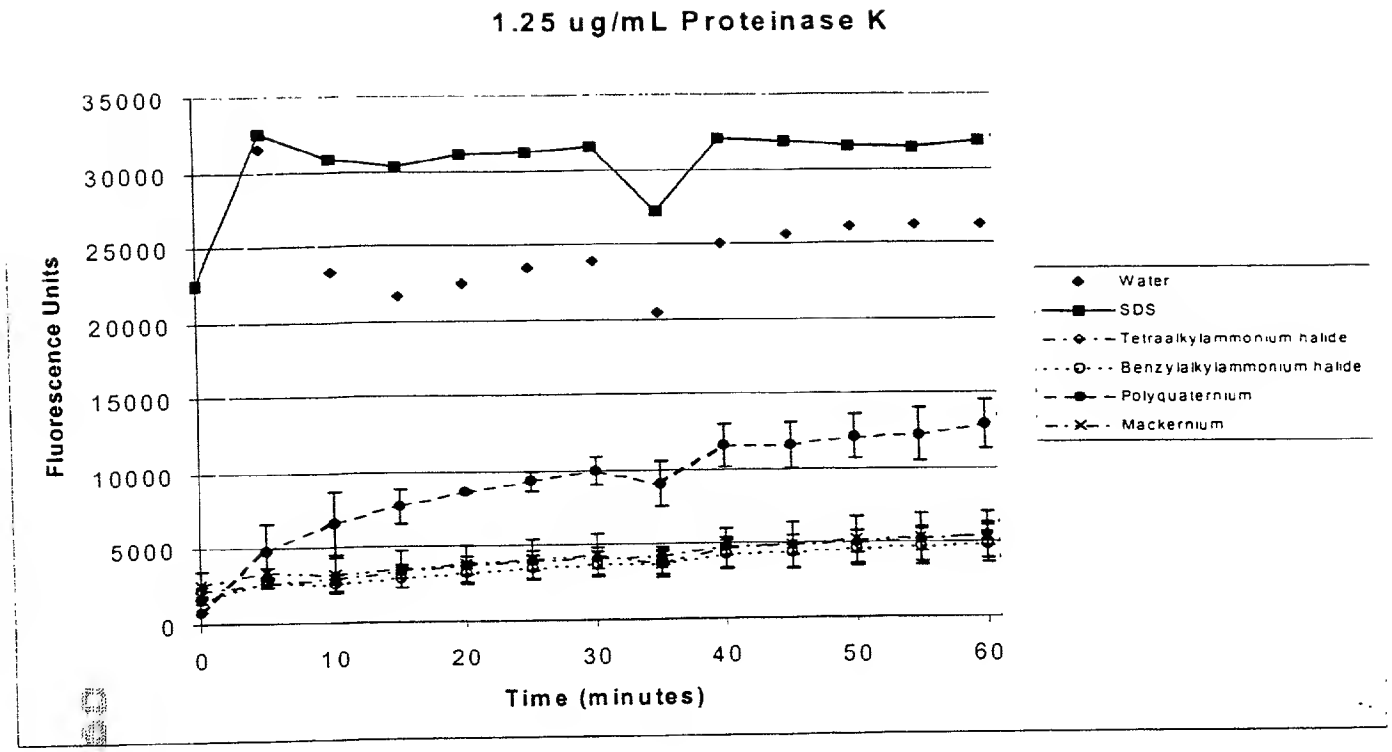

1.25 ug/mL Proteinase K
35000
30000
25000
20000
15000
10000
5000
0
Fluorescence Units
0
10
20
30
40
50
60
Time (minutes)
Water
SDS
Tetraalkylammonium halide
Benzylalkylammonium halide
Polyquaternium
Mackernium

Figure 2C

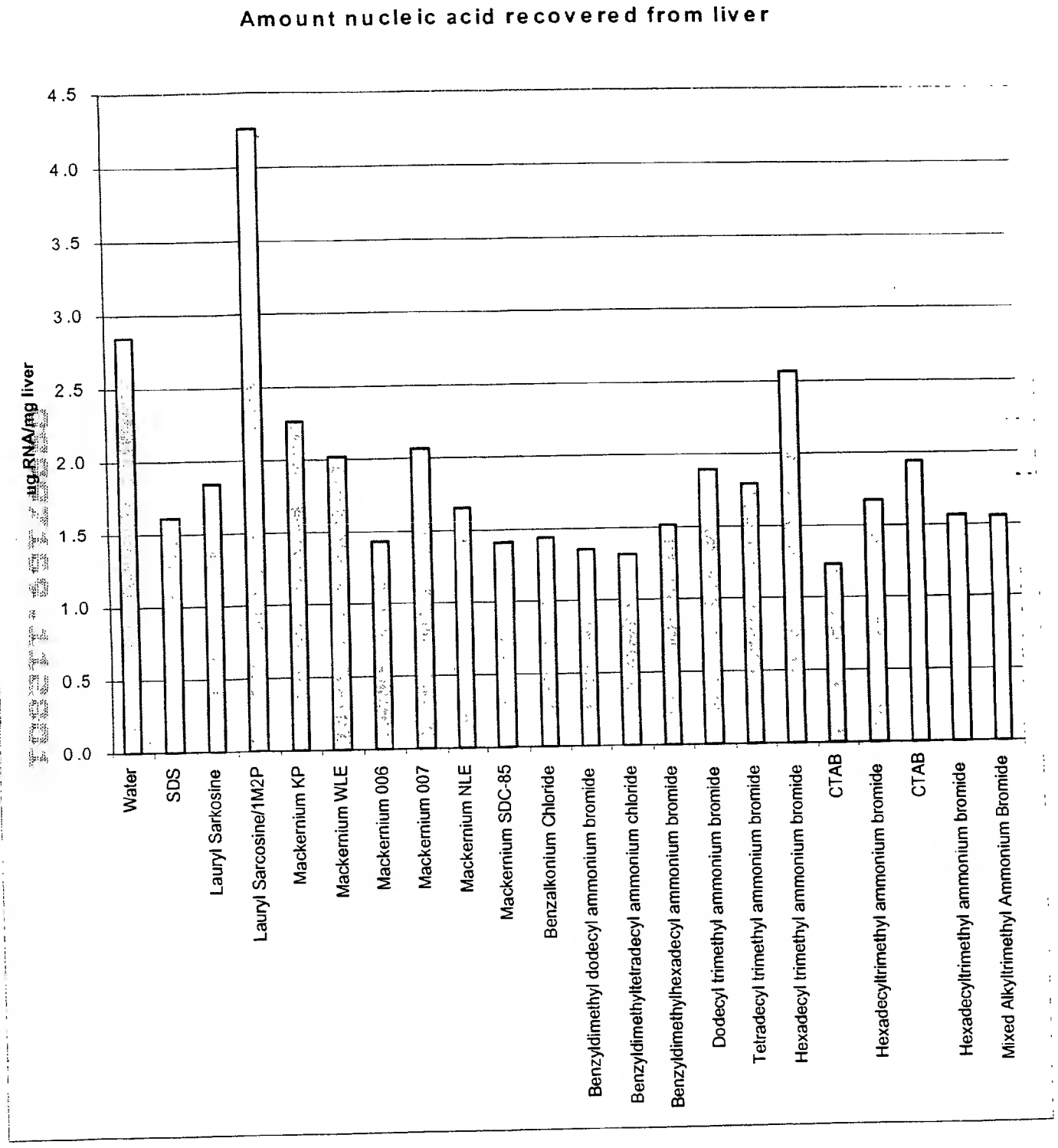

Amount nucleic acid recovered from liver
ug RNA/mg liver
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Water
SDS
Lauryl Sarkosine
Lauryl Sarcosine/1M2P
Mackernium KP
Mackernium WLE
Mackernium 006
Mackernium 007
Mackernium NLE
Mackernium SDC-85
Benzalkonium Chloride
Benzyldimethyl dodecyl ammonium bromide
Benzyldimethyltetradecyl ammonium chloride
Benzyldimethylhexadecyl ammonium bromide
Dodecyl trimethyl ammonium bromide
Tetradecyl trimethyl ammonium bromide
Hexadecyl trimethyl ammonium bromide
CTAB
Hexadecyltrimethyl ammonium bromide
CTAB
Hexadecyltrimethyl ammonium bromide
Mixed Alkyltrimethyl Ammonium Bromide

Figure 3

1 KB standard
RNA Ladder
Control RNA
Supe: Water
Supe: SDS
Supe: Mackernium KP
Supe: Mackernium WLE
Supe: Benzalonium Chloride
Supe: Mackernium 006
Supe: Mackernium 007
Supe: Mackernium NLE
Supe: Dodecyl trimethyl ammonium bromide
Supe: Tetradecyl trimethyl ammonium bromide
Supe: Hexadecyl trimethyl ammonium bromide
Supe: CTAB
Supe: Mackernium SDC-85
Supe: Mixed Alkyl trimethyl ammonium bromde (C_{14})
Supe: Benzyl dimethyl ammonium bromide
Supe: Benzyl dimethyl hexadecyl ammonium chloride
Supe: Benzyldimethyl tetradecyl ammonium chloride

Figure 4

Figure 4 (cont.)

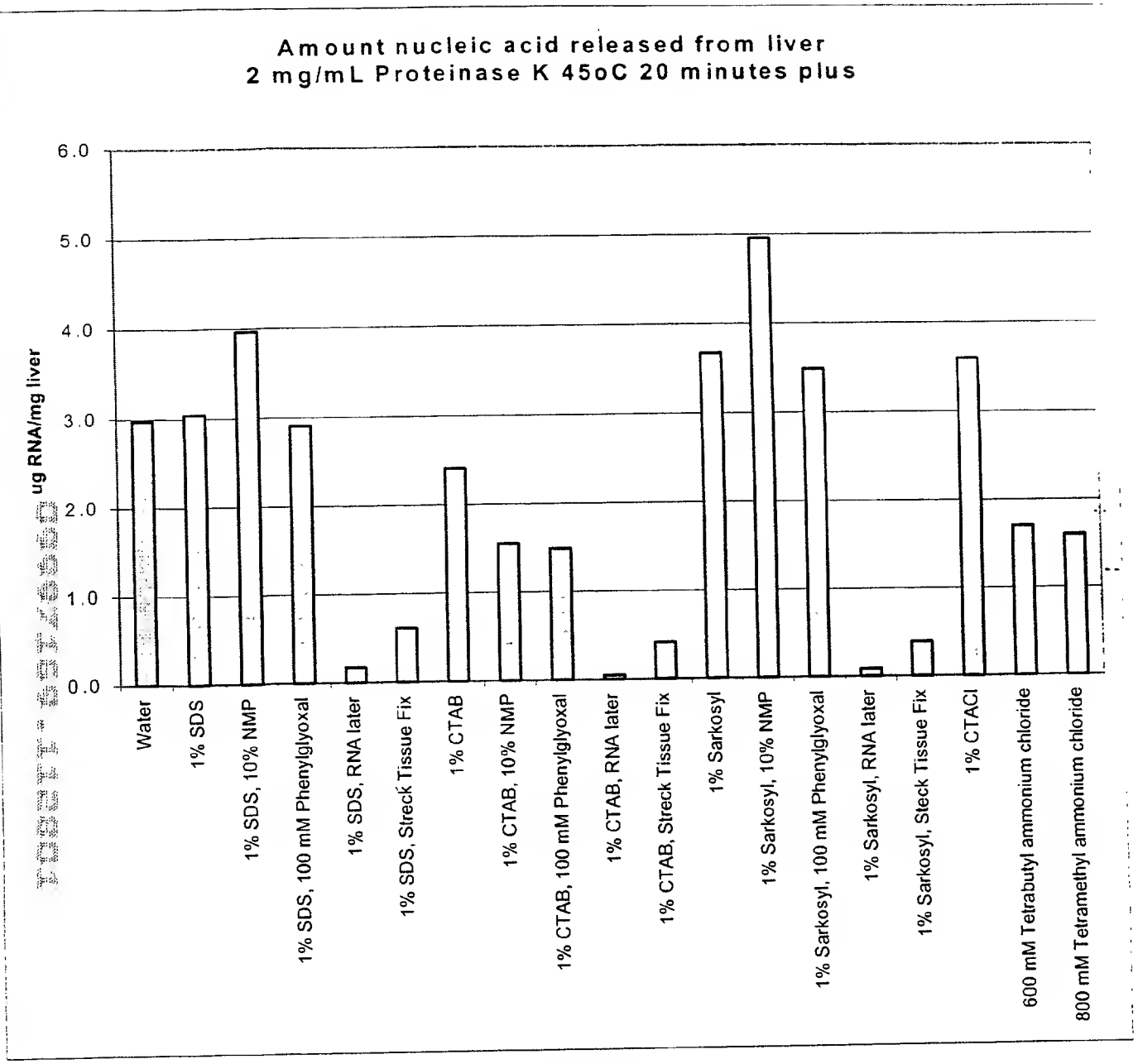

Amount nucleic acid released from liver
2 mg/mL Proteinase K 45oC 20 minutes plus
ug RNA/mg liver
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Water
1% SDS
1% SDS, 10% NMP
1% SDS, 100 mM Phenylglyoxal
1% SDS, RNA later
1% SDS, Streck Tissue Fix
1% CTAB
1% CTAB, 10% NMP
1% CTAB, 100 mM Phenylglyoxal
1% CTAB, RNA later
1% CTAB, Streck Tissue Fix
1% Sarkosyl
1% Sarkosyl, 10% NMP
1% Sarkosyl, 100 mM Phenylglyoxal
1% Sarkosyl, RNA later
1% Sarkosyl, Steck Tissue Fix
1% CTACl
600 mM Tetrabutyl ammonium chloride
800 mM Tetramethyl ammonium chloride

Figure 5

1KB DNA Standard
RNA Ladder
Human RNA control
No detergent
1% SDS
10% 1 Methyl 2-pyrolidinone
1% SDS
100 mM phenylglyoxal
1% SDS
RNA Later
1% SDS
Streck Tissue Fixative
1% SDS
1% CTAB
10% 1 Methyl 2-pyrolidinone
1% CTAB
100 mM phenylglyoxal
1% CTAB
RNA Later
1% CTAB
Streck Tissue Fixative
1% CTAB
1% Sarkosyl
10% 1 Methyl 2-pyrolidinone
1% Sarkosyl
100 mM phenylglyoxal
1% Sarkosyl
RNA Later
1% Sarkosyl
Streck Tissue Fixative
1% Sarkosyl
1% CTACl
600 mM tetrabutyl ammonium
No detergent
800 mM tetramethyl
No detergent

Figure 6

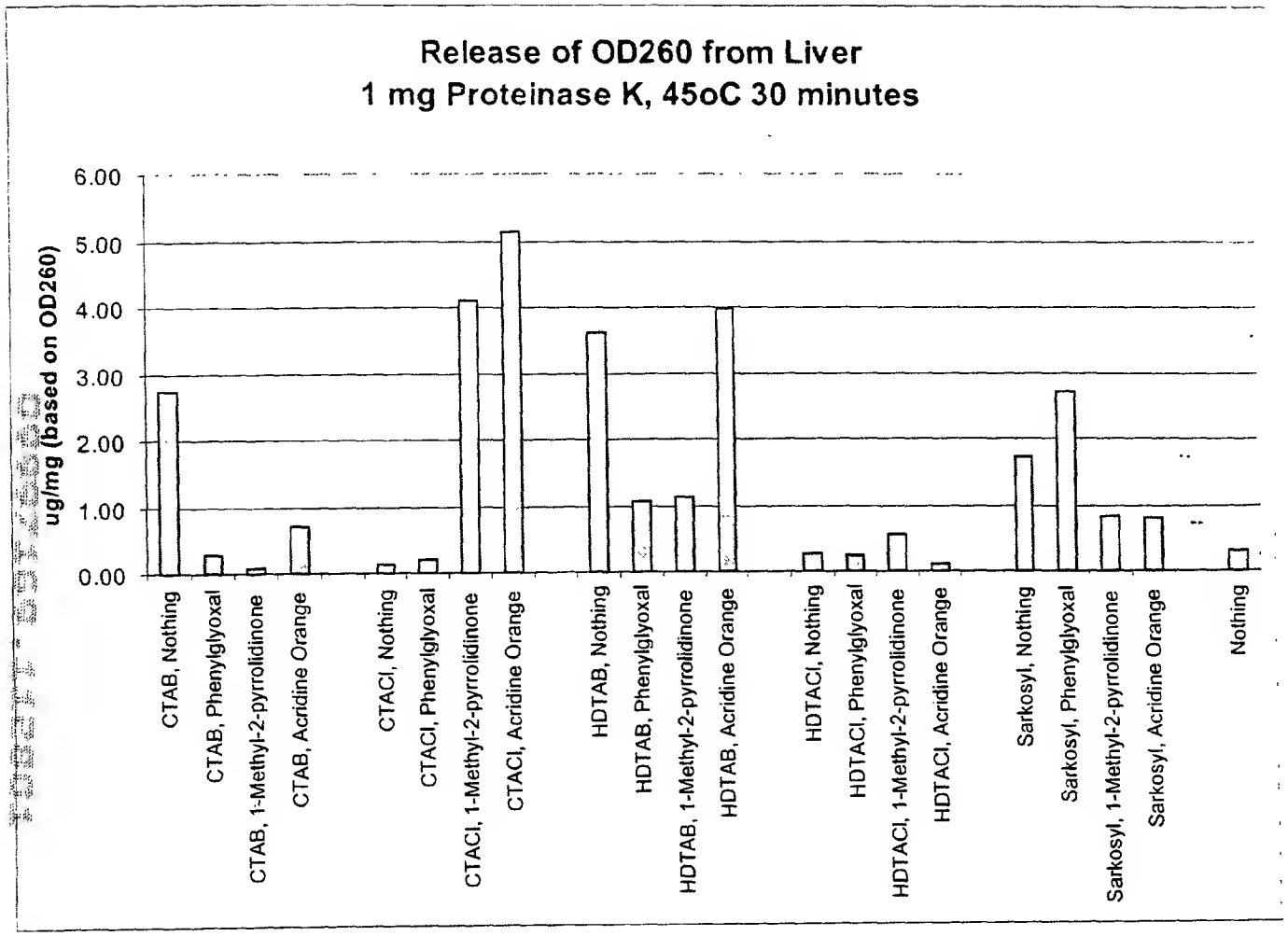
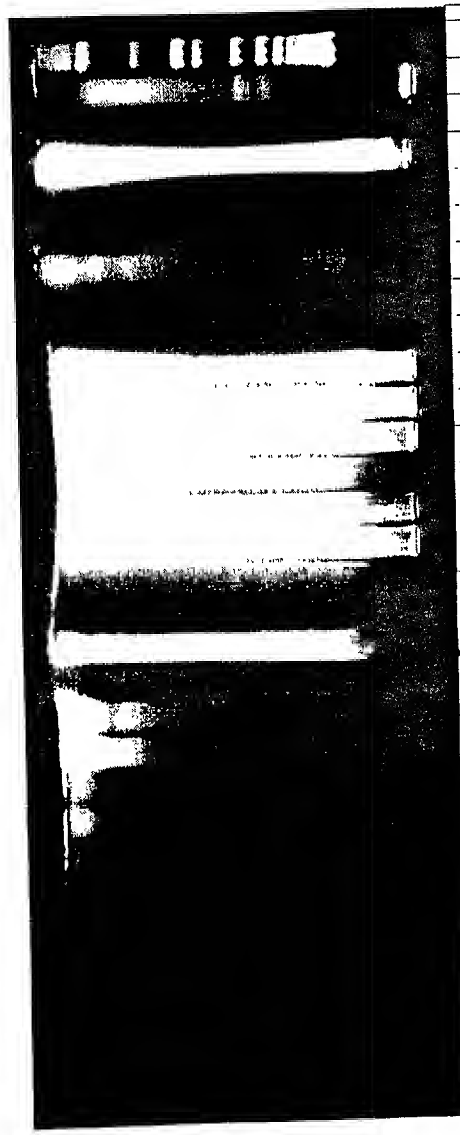

Release of OD260 from Liver
1 mg Proteinase K, 45oC 30 minutes
ug/mg (based on OD260)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
CTAB, Nothing
CTAB, Phenylglyoxal
CTAB, 1-Methyl-2-pyrrolidinone
CTAB, Acridine Orange
CTACl, Nothing
CTACl, Phenylglyoxal
CTACl, 1-Methyl-2-pyrrolidinone
CTACl, Acridine Orange
HDTAB, Nothing
HDTAB, Phenylglyoxal
HDTAB, 1-Methyl-2-pyrrolidinone
HDTAB, Acridine Orange
HDTACl, Nothing
HDTACl, Phenylglyoxal
HDTACl, 1-Methyl-2-pyrrolidinone
HDTACl, Acridine Orange
Sarkosyl, Nothing
Sarkosyl, Phenylglyoxal
Sarkosyl, 1-Methyl-2-pyrrolidinone
Sarkosyl, Acridine Orange
Nothing

Figure 7



None	Cetyltrimethylammonium bromide
phenylglyoxal	Cetyltrimethylammonium bromide
1-methyl-2-pyrorolidinone	Cetyltrimethylammonium bromide
Acridine Orange	Cetyltrimethylammonium bromide
None	Cetyltrimethylammonium chloride
phenylglyoxal	Cetyltrimethylammonium chloride
1-methyl-2-pyrorolidinone	Cetyltrimethylammonium chloride
Acridine Orange	Cetyltrimethylammonium chloride
None	Hexadecyltrimethlammonium bromide
phenylglyoxal	Hexadecyltrimethlammonium bromide
1-methyl-2-pyrorolidinone	Hexadecyltrimethlammonium bromide
Acridine Orange	Hexadecyltrimethlammonium bromide
None	Hexadecyltrimethlammonium chloride
phenylglyoxal	Hexadecyltrimethlammonium chloride
1-methyl-2-pyrorolidinone	Hexadecyltrimethlammonium chloride
Acridine Orange	Hexadecyltrimethlammonium chloride
None	Sarkosyl
phenylglyoxal	Sarkosyl
1-methyl-2-pyrorolidinone	Sarkosyl
Acridine Orange	Sarkosyl
	No detergent

Figure 8

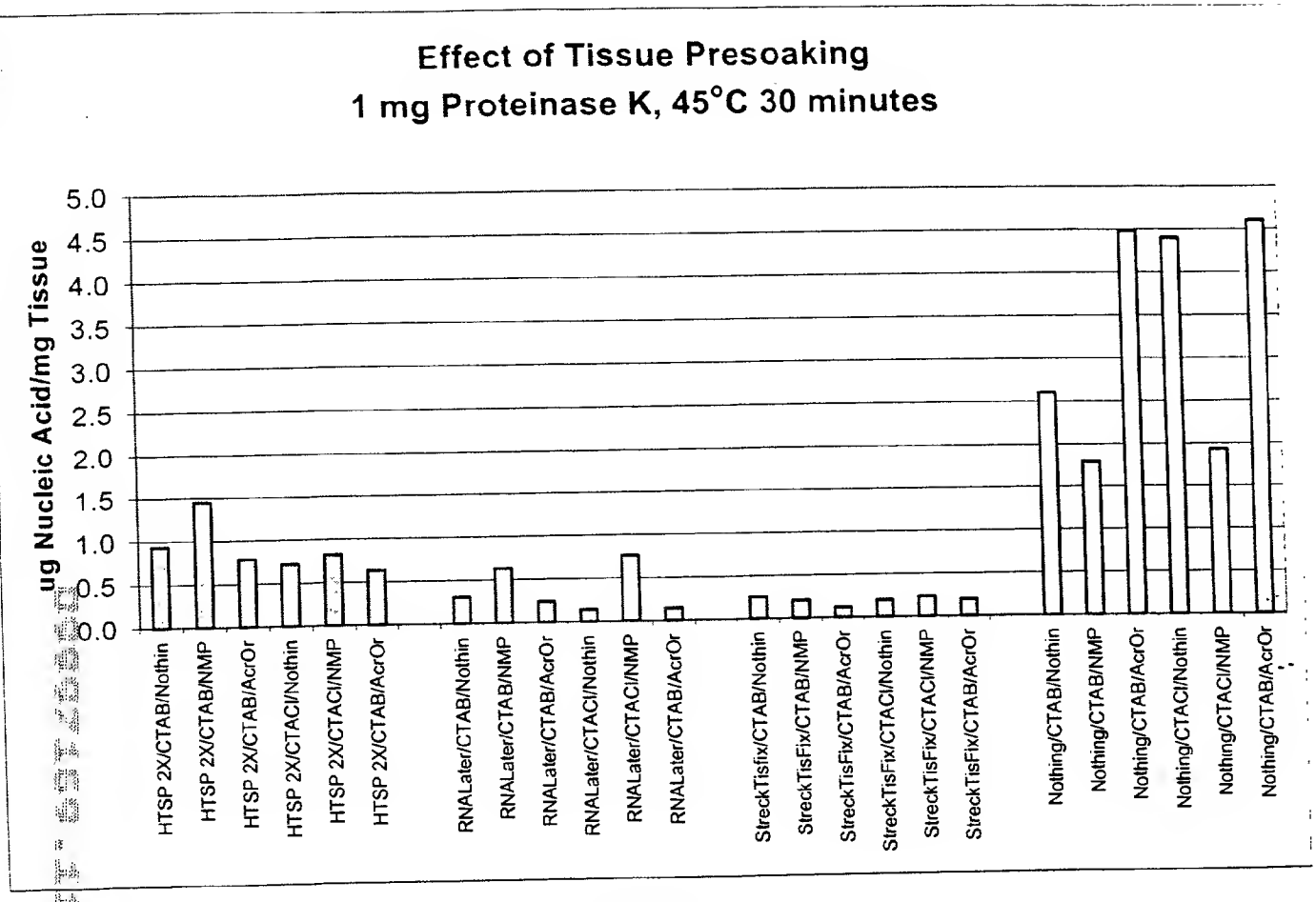

Effect of Tissue Presoaking
1 mg Proteinase K, 45°C 30 minutes
ug Nucleic Acid/mg Tissue
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HTSP 2X/CTAB/Nothin
HTSP 2X/CTAB/NMP
HTSP 2X/CTAB/AcrOr
HTSP 2X/CTACl/Nothin
HTSP 2X/CTACl/NMP
HTSP 2X/CTAB/AcrOr
RNALater/CTAB/Nothin
RNALater/CTAB/NMP
RNALater/CTAB/AcrOr
RNALater/CTACl/Nothin
RNALater/CTACl/NMP
RNALater/CTAB/AcrOr
StreckTisfix/CTAB/Nothin
StreckTisFix/CTAB/NMP
StreckTisFix/CTAB/AcrOr
StreckTisFix/CTACl/Nothin
StreckTisFix/CTACl/NMP
StreckTisFix/CTAB/AcrOr
Nothing/CTAB/Nothin
Nothing/CTAB/NMP
Nothing/CTAB/AcrOr
Nothing/CTACl/Nothin
Nothing/CTACl/NMP
Nothing/CTAB/AcrOr

Figure 9

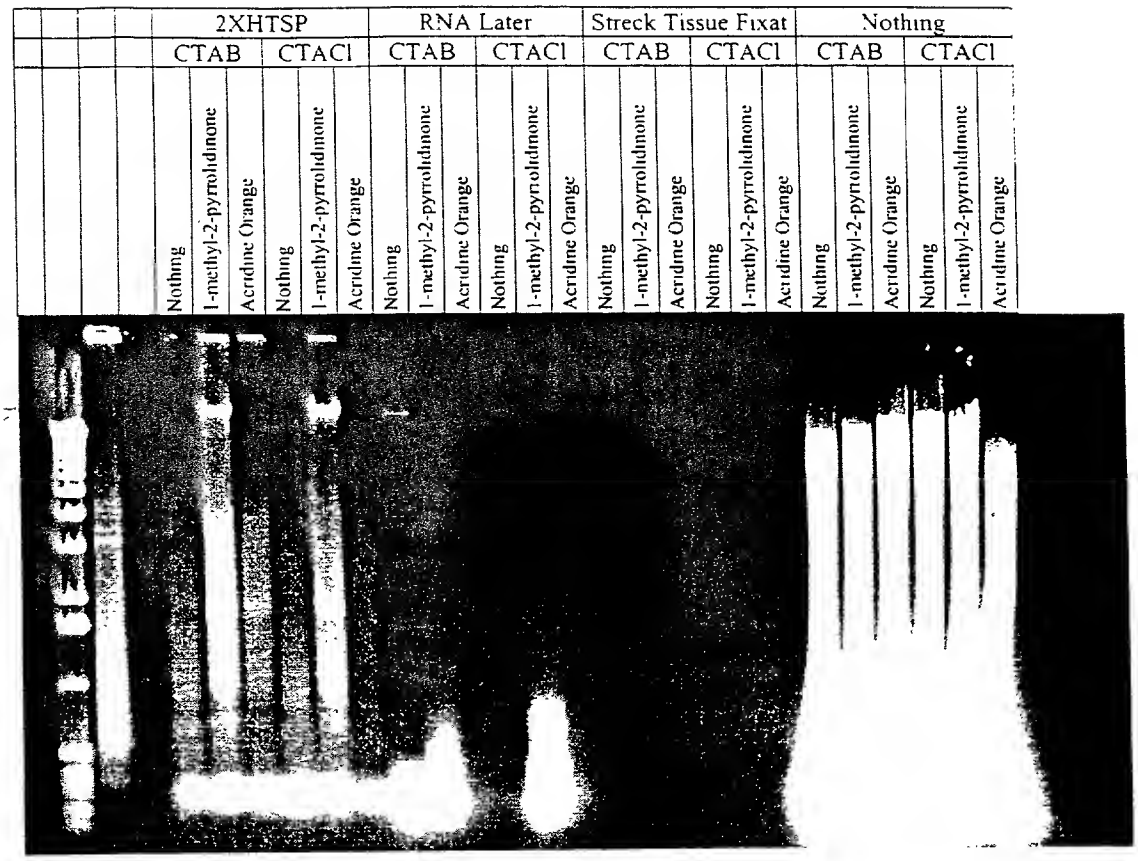

2XHTSP
RNA Later
Streck Tissue Fixat
Nothing
CTAB
CTACl
Nothing
1-methyl-2-pyrrolidinone
Acridine Orange

Figure 10

1% CTAB
5 mM Aurintricarboyxlic Acid
2 mM Aurintricarboxylic Acid
1 mM Aurintricarboyxlic Acid
0.5 mM Aurintricarboyxlic Acid
0.2 mM Aurintricarboyxlic Acid
0.1 mM Aurintricarboyxlic Acid
1% CTACl
5 mM Aurintricarboyxlic Acid
2 mM Aurintricarboxylic Acid
1 mM Aurintricarboyxlic Acid
0.5 mM Aurintricarboyxlic Acid
0.2 mM Aurintricarboyxlic Acid
0.1 mM Aurintricarboyxlic Acid
1% SDS
5 mM Aurintricarboyxlic Acid
2 mM Aurintricarboxylic Acid
1 mM Aurintricarboyxlic Acid
0.5 mM Aurintricarboyxlic Acid
0.2 mM Aurintricarboyxlic Acid
0.1 mM Aurintricarboyxlic Acid

Figure 11

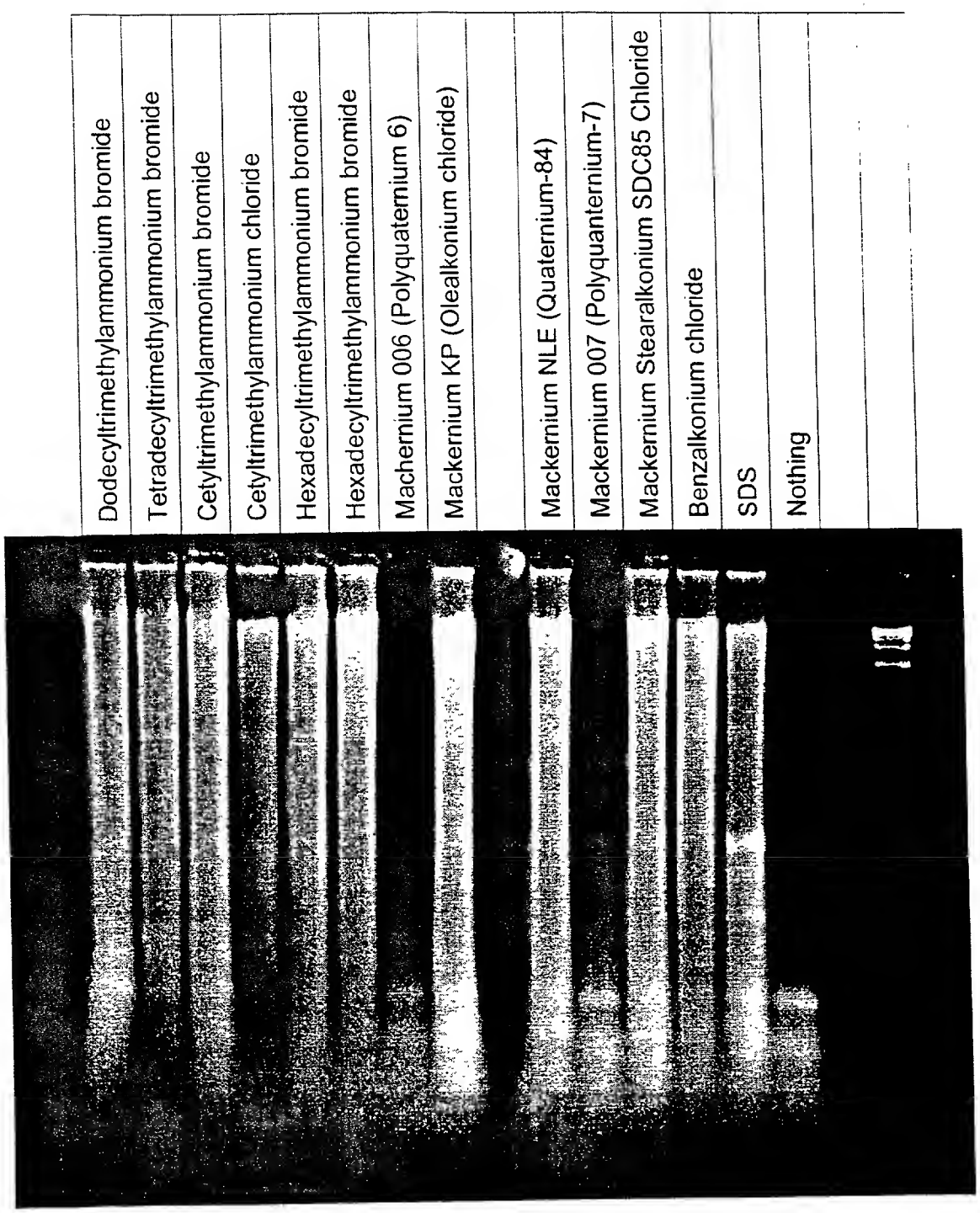

Dodecyltrimethylammonium bromide
Tetradecyltrimethylammonium bromide
Cetyltrimethylammonium bromide
Cetyltrimethylammonium chloride
Hexadecyltrimethylammonium bromide
Hexadecyltrimethylammonium bromide
Machernium 006 (Polyquaternium 6)
Mackernium KP (Olealkonium chloride)
Mackernium NLE (Quaternium-84)
Mackernium 007 (Polyquanternium-7)
Mackernium Stearalkonium SDC85 Chloride
Benzalkonium chloride
SDS
Nothing

Figure 12

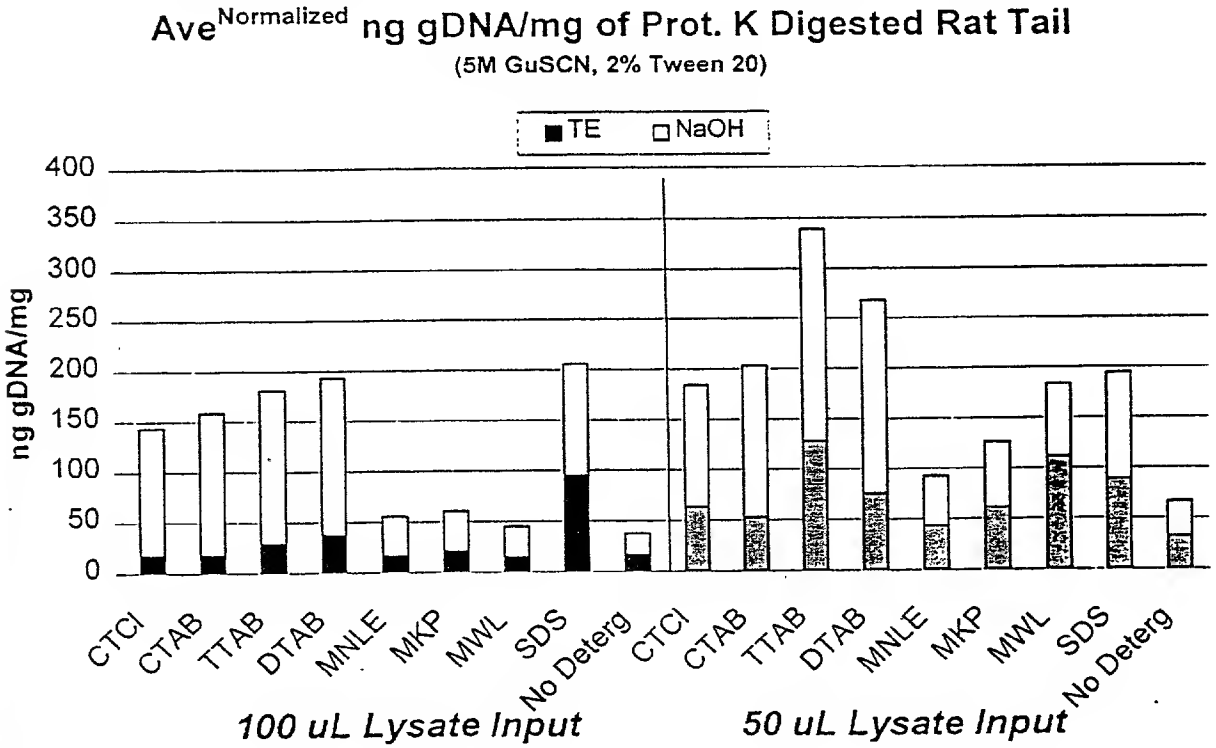

Ave Normalized ng gDNA/mg of Prot. K Digested Rat Tail
(5M GuSCN, 2% Tween 20)
TE
NaOH
ng gDNA/mg
400
350
300
250
200
150
100
50
0
CTCl
CTAB
TTAB
DTAB
MNLE
MKP
MWL
SDS
No Deterg
100 uL Lysate Input
50 uL Lysate Input

Figure 13

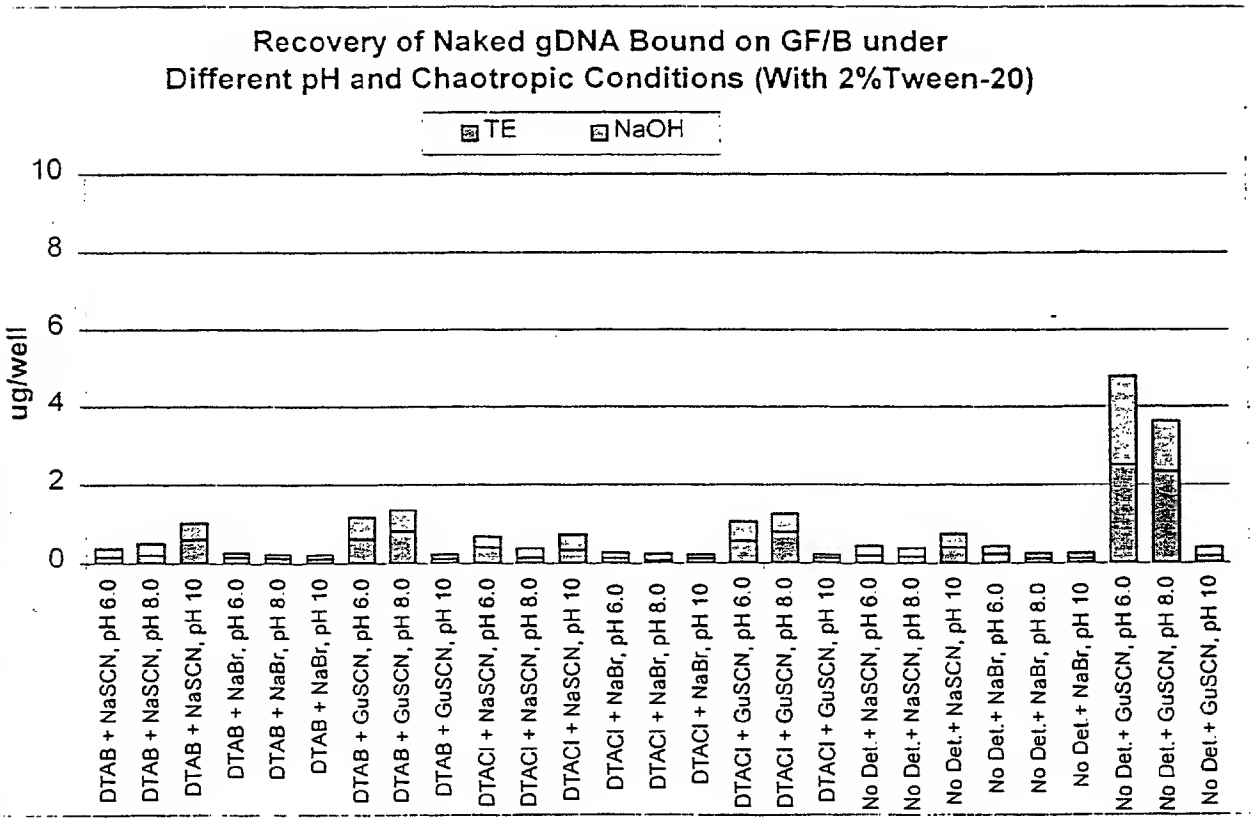

Recovery of Naked gDNA Bound on GF/B under
Different pH and Chaotropic Conditions (With 2%Tween-20)
TE
NaOH
ug/well
10
8
6
4
2
0
DTAB + NaSCN, pH 6.0
DTAB + NaSCN, pH 8.0
DTAB + NaSCN, pH 10
DTAB + NaBr, pH 6.0
DTAB + NaBr, pH 8.0
DTAB + NaBr, pH 10
DTAB + GuSCN, pH 6.0
DTAB + GuSCN, pH 8.0
DTAB + GuSCN, pH 10
DTACl + NaSCN, pH 6.0
DTACl + NaSCN, pH 8.0
DTACl + NaSCN, pH 10
DTACl + NaBr, pH 6.0
DTACl + NaBr, pH 8.0
DTACl + NaBr, pH 10
DTACl + GuSCN, pH 6.0
DTACl + GuSCN, pH 8.0
DTACl + GuSCN, pH 10
No Det.+ NaSCN, pH 6.0
No Det.+ NaSCN, pH 8.0
No Det.+ NaSCN, pH 10
No Det.+ NaBr, pH 6.0
No Det.+ NaBr, pH 8.0
No Det.+ NaBr, pH 10
No Det.+ GuSCN, pH 6.0
No Det.+ GuSCN, pH 8.0
No Det.+ GuSCN, pH 10

Figure 14

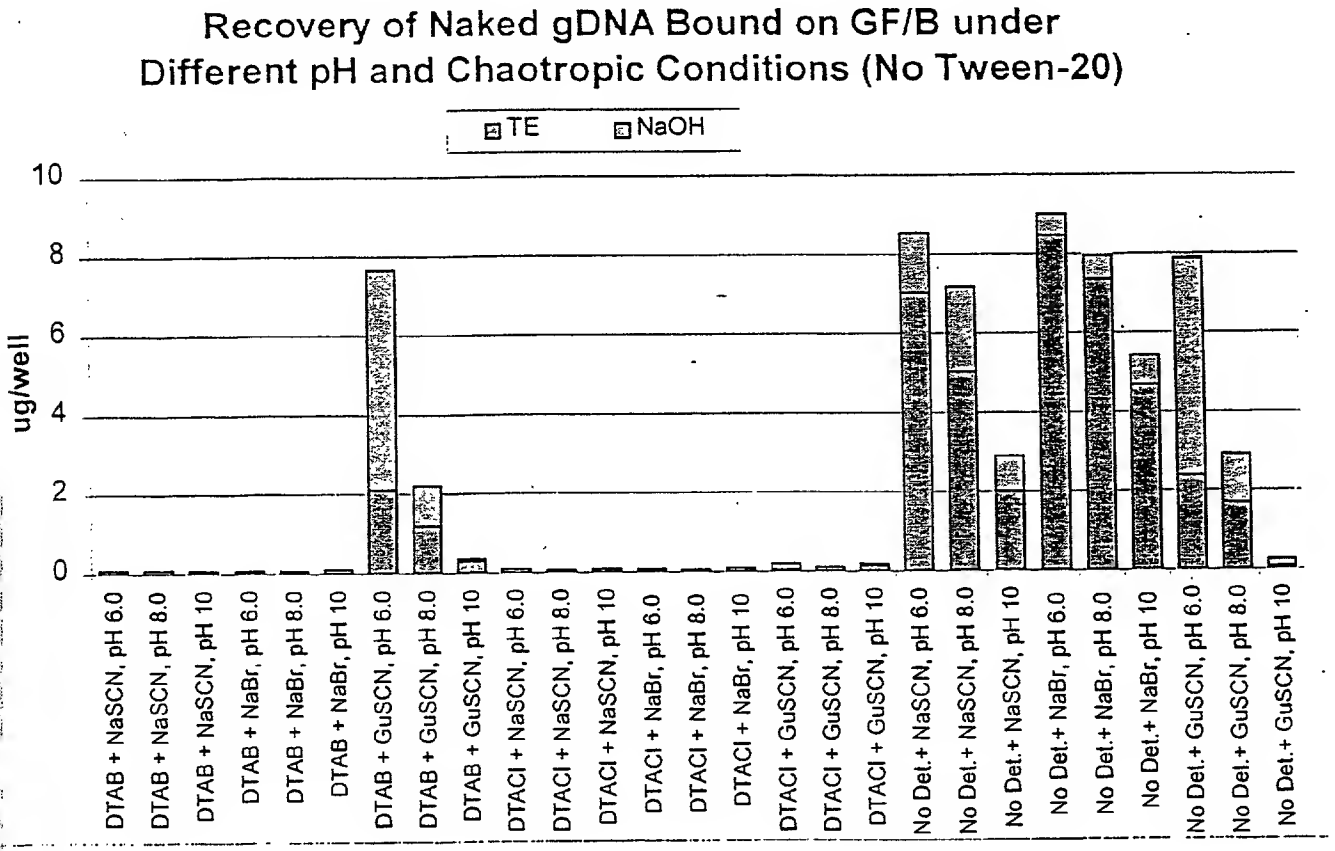

Recovery of Naked gDNA Bound on GF/B under Different pH and Chaotropic Conditions (No Tween-20)
TE
NaOH
ug/well
10
8
6
4
2
0
DTAB + NaSCN, pH 6.0
DTAB + NaSCN, pH 8.0
DTAB + NaSCN, pH 10
DTAB + NaBr, pH 6.0
DTAB + NaBr, pH 8.0
DTAB + NaBr, pH 10
DTAB + GuSCN, pH 6.0
DTAB + GuSCN, pH 8.0
DTAB + GuSCN, pH 10
DTACl + NaSCN, pH 6.0
DTACl + NaSCN, pH 8.0
DTACl + NaSCN, pH 10
DTACl + NaBr, pH 6.0
DTACl + NaBr, pH 8.0
DTACl + NaBr, pH 10
DTACl + GuSCN, pH 6.0
DTACl + GuSCN, pH 8.0
DTACl + GuSCN, pH 10
No Det.+ NaSCN, pH 6.0
No Det.+ NaSCN, pH 8.0
No Det.+ NaSCN, pH 10
No Det.+ NaBr, pH 6.0
No Det.+ NaBr, pH 8.0
No Det.+ NaBr, pH 10
No Det.+ GuSCN, pH 6.0
No Det.+ GuSCN, pH 8.0
No Det.+ GuSCN, pH 10

Figure 15

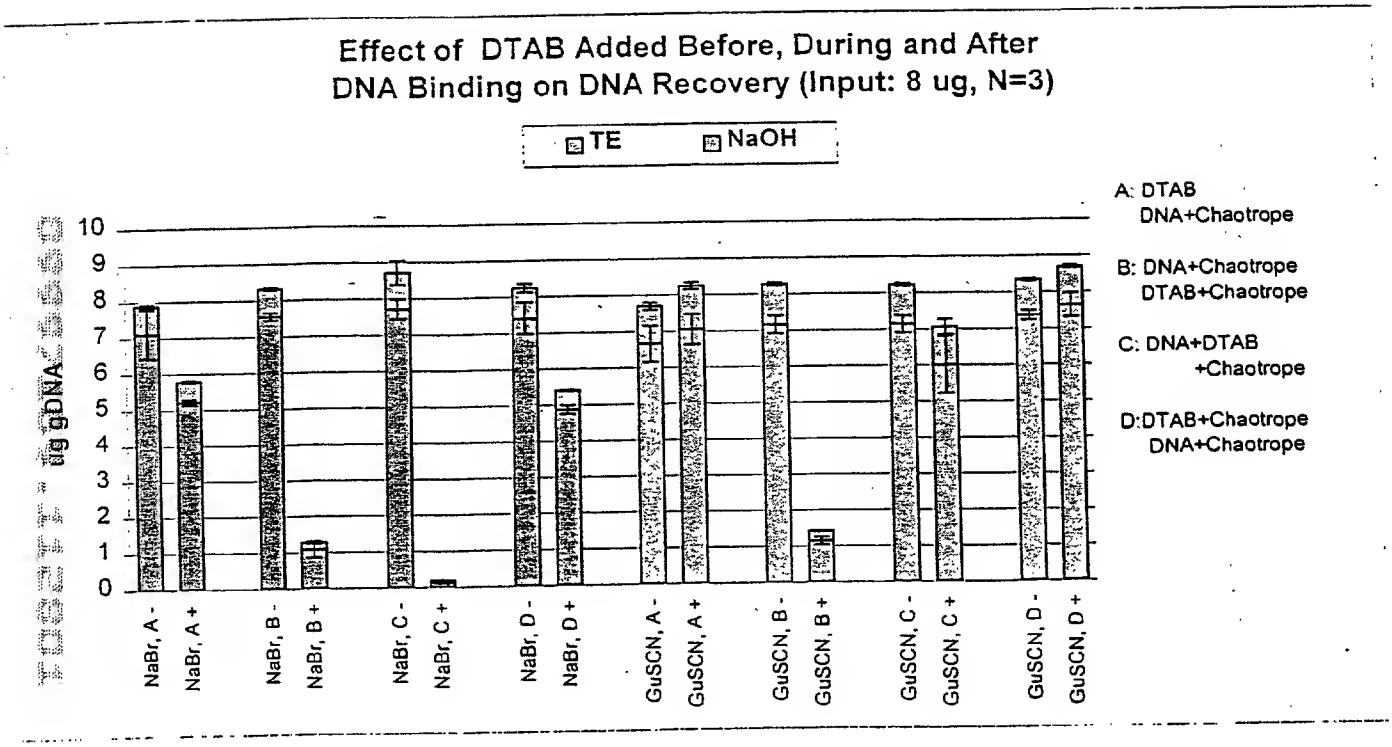

Effect of DTAB Added Before, During and After
DNA Binding on DNA Recovery (Input: 8 ug, N=3)
TE
NaOH
ug gDNA / 50 ul
10
9
8
7
6
5
4
3
2
1
0
NaBr, A -
NaBr, A +
NaBr, B -
NaBr, B +
NaBr, C -
NaBr, C +
NaBr, D -
NaBr, D +
GuSCN, A -
GuSCN, A +
GuSCN, B -
GuSCN, B +
GuSCN, C -
GuSCN, C +
GuSCN, D -
GuSCN, D +
A: DTAB
DNA+Chaotrope
B: DNA+Chaotrope
DTAB+Chaotrope
C: DNA+DTAB
+Chaotrope
D:DTAB+Chaotrope
DNA+Chaotrope

Figure 16

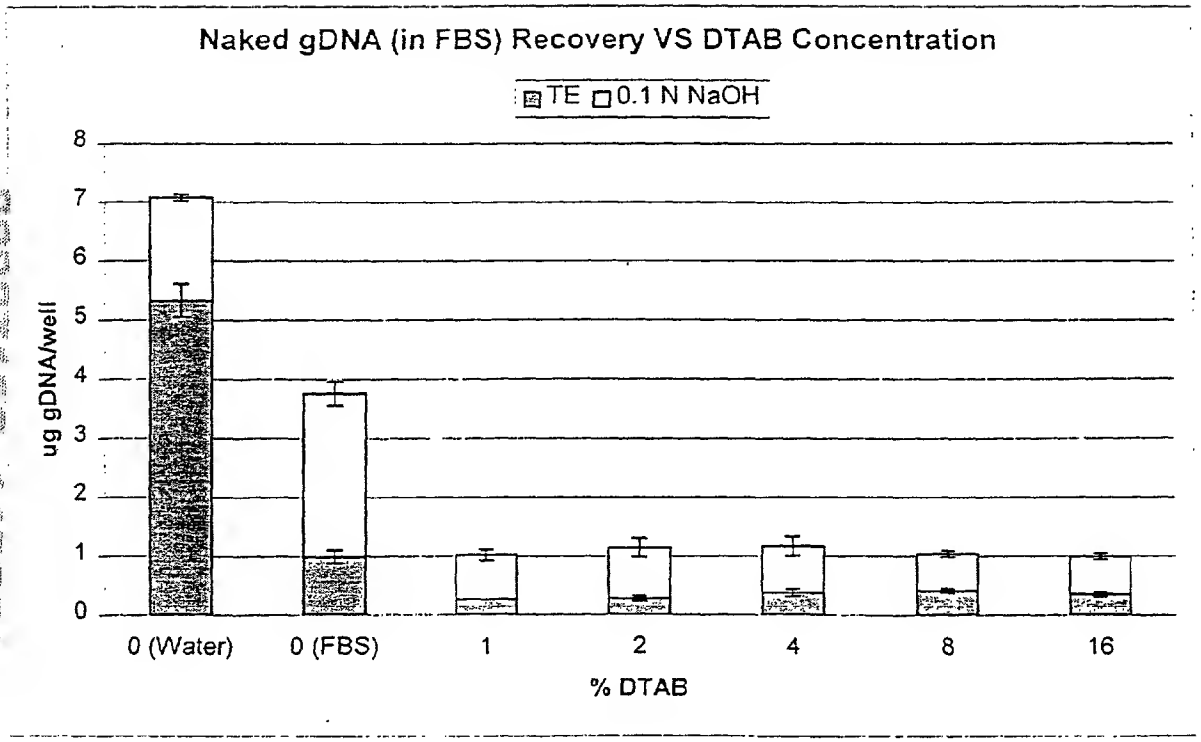

Naked gDNA (in FBS) Recovery VS DTAB Concentration
TE
0.1 N NaOH
ug gDNA/well
0
1
2
3
4
5
6
7
8
0 (Water)
0 (FBS)
1
2
4
8
16
% DTAB

Figure 17

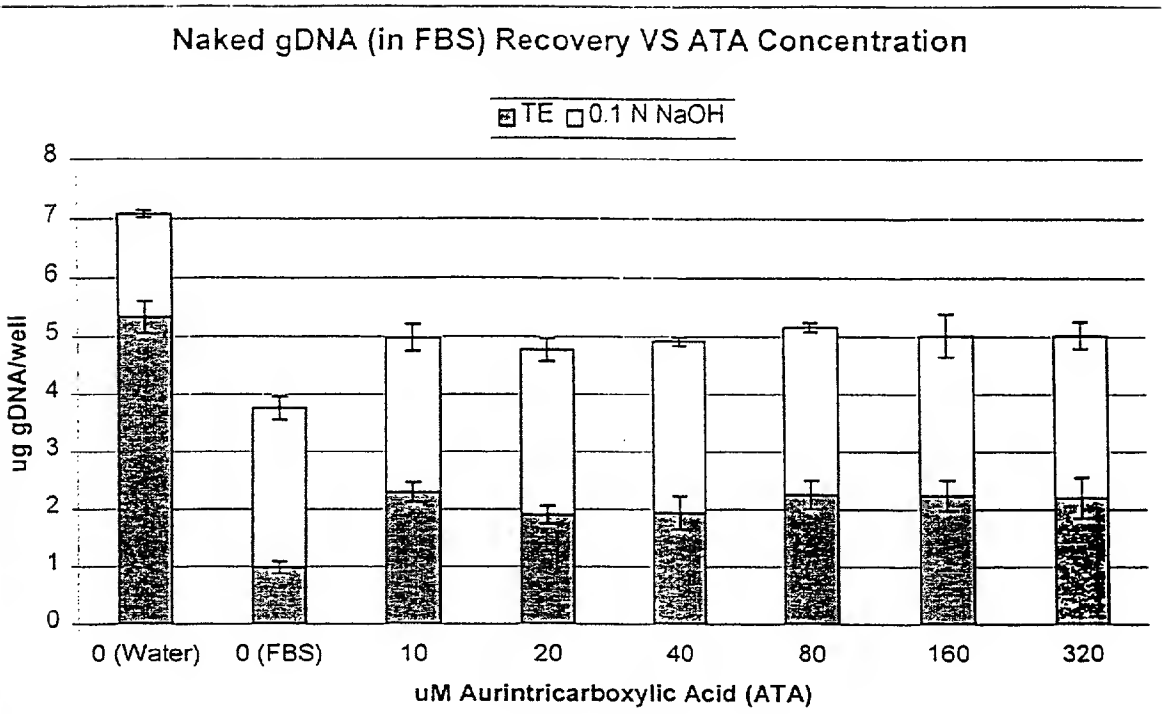

Naked gDNA (in FBS) Recovery VS ATA Concentration
TE
0.1 N NaOH
ug gDNA/well
0
1
2
3
4
5
6
7
8
0 (Water)
0 (FBS)
10
20
40
80
160
320
uM Aurintricarboxylic Acid (ATA)

Figure 18

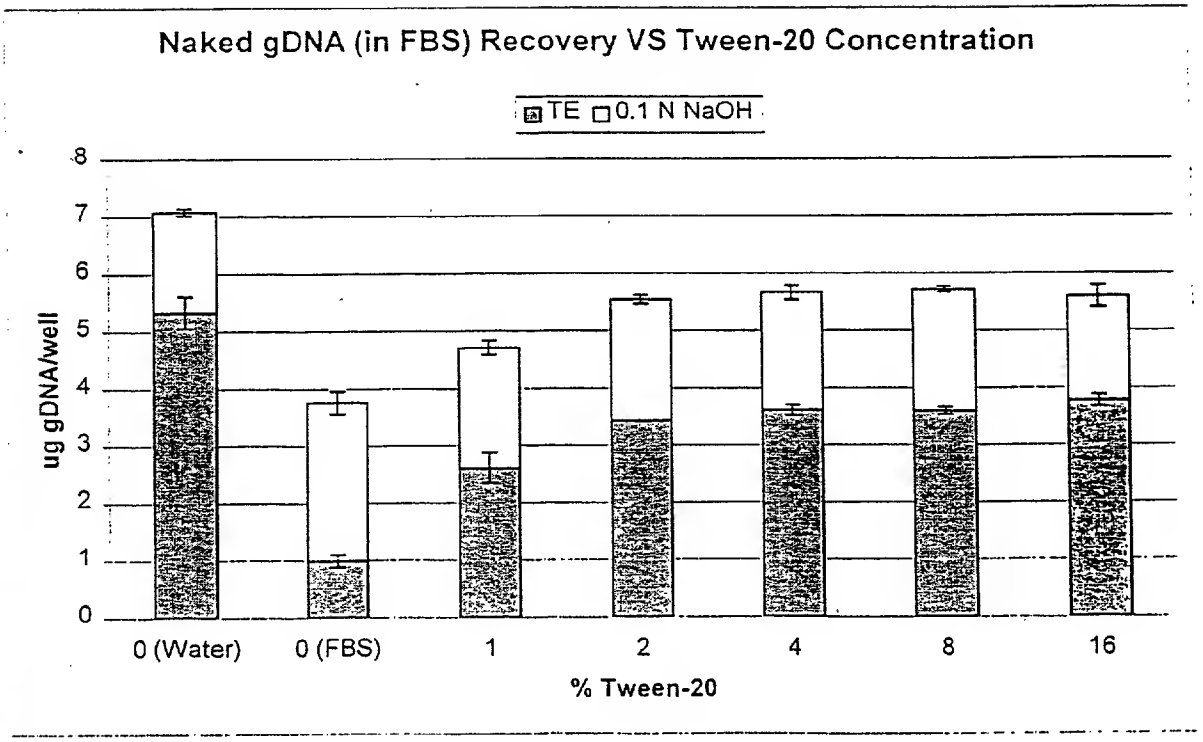

Naked gDNA (in FBS) Recovery VS Tween-20 Concentration
TE
0.1 N NaOH
ug gDNA/well
0
1
2
3
4
5
6
7
8
0 (Water)
0 (FBS)
1
2
4
8
16
% Tween-20

Figure 19

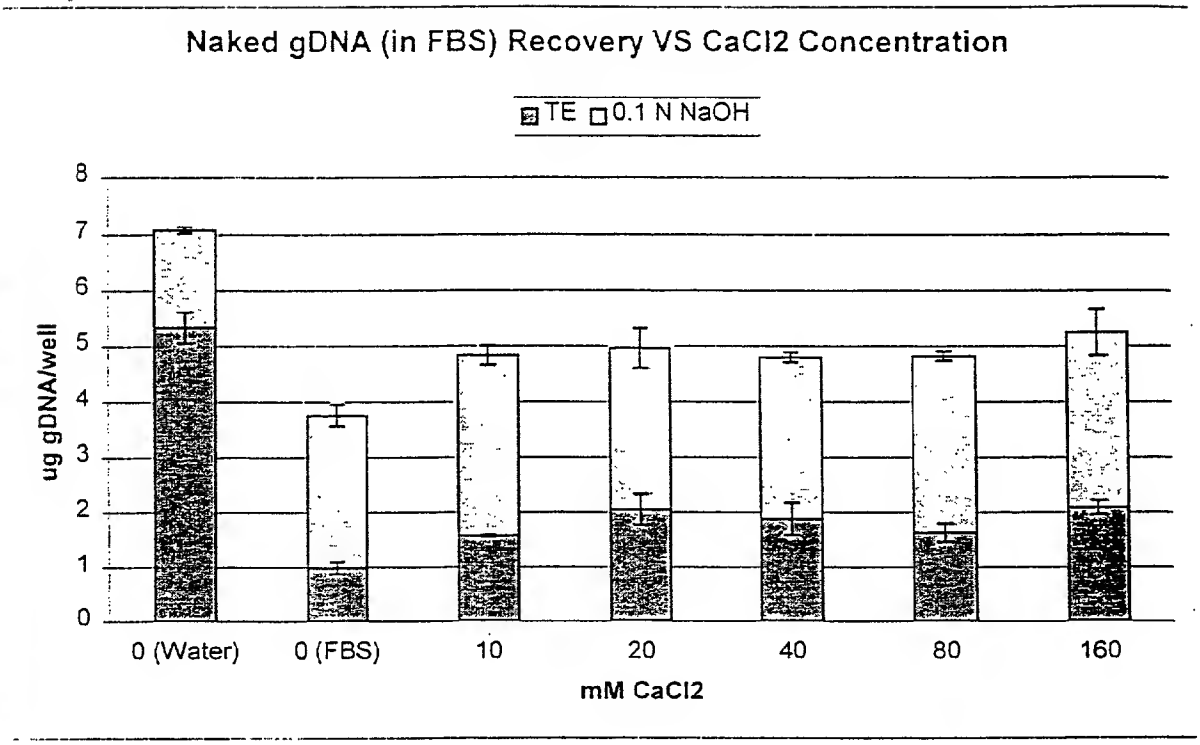

Naked gDNA (in FBS) Recovery VS CaCl2 Concentration
TE
0.1 N NaOH
ug gDNA/well
0
1
2
3
4
5
6
7
8
0 (Water)
0 (FBS)
10
20
40
80
160
mM CaCl2

Figure 20

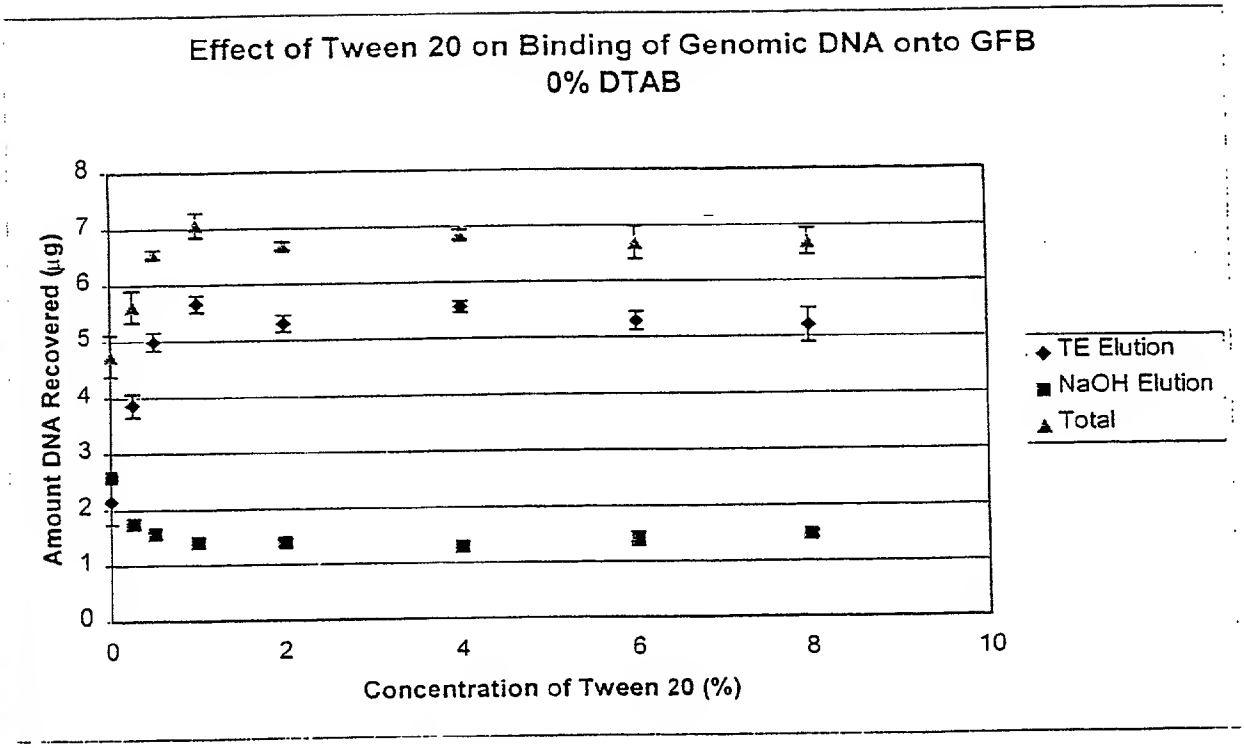

Effect of Tween 20 on Binding of Genomic DNA onto GFB
0% DTAB
Amount DNA Recovered (µg)
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
Concentration of Tween 20 (%)
TE Elution
NaOH Elution
Total

Figure 21

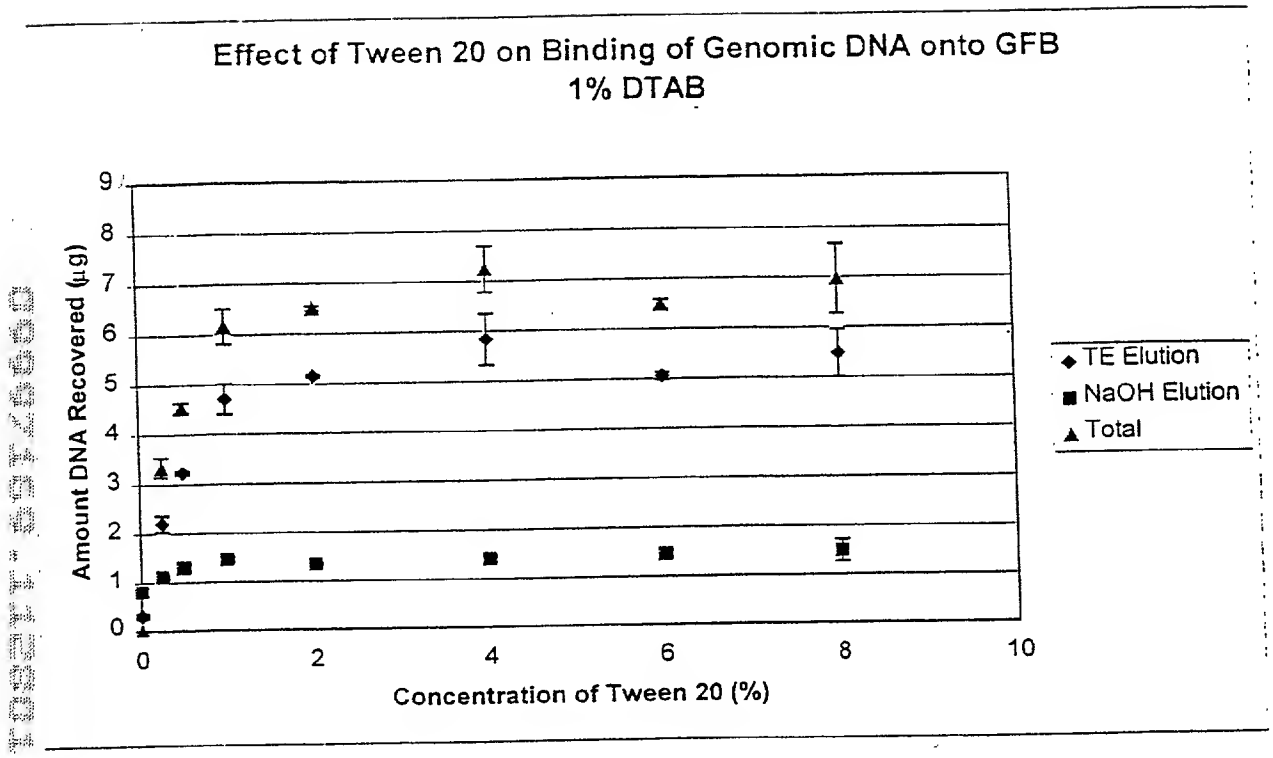

Effect of Tween 20 on Binding of Genomic DNA onto GFB
1% DTAB
Amount DNA Recovered (µg)
0
1
2
3
4
5
6
7
8
9
0
2
4
6
8
10
Concentration of Tween 20 (%)
TE Elution
NaOH Elution
Total

Figure 22

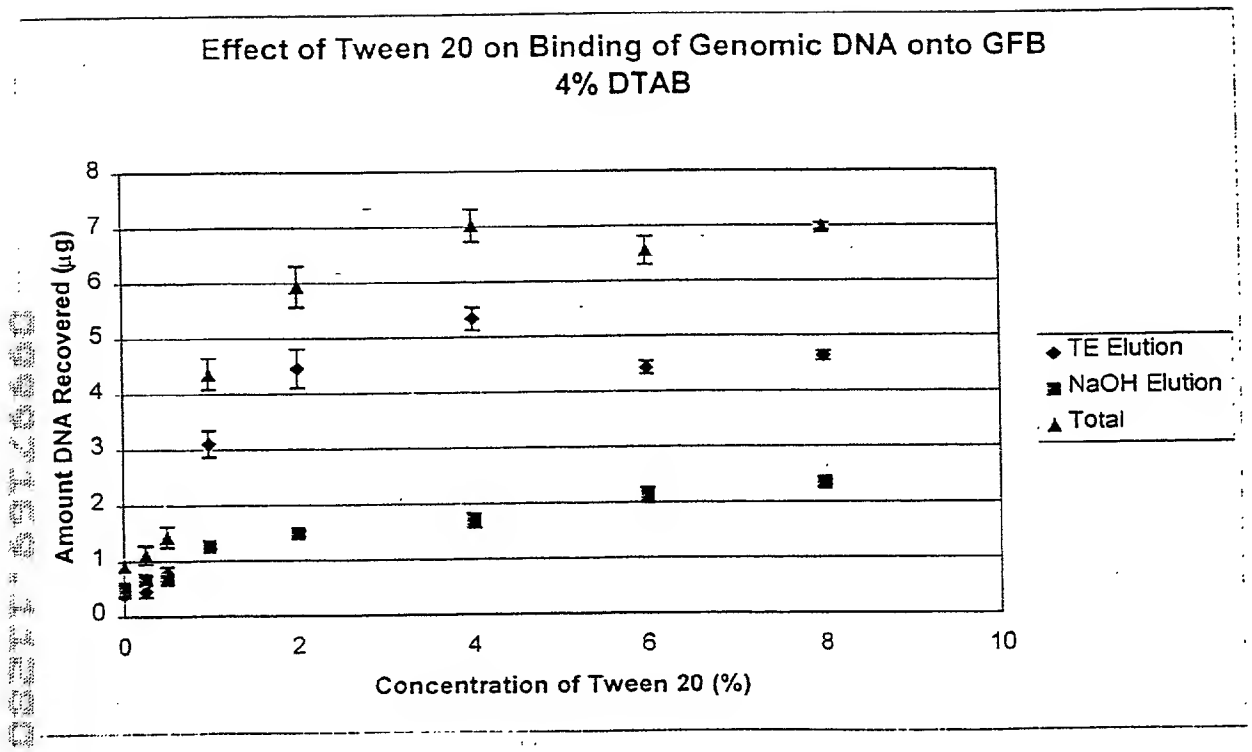

Effect of Tween 20 on Binding of Genomic DNA onto GFB
4% DTAB
Amount DNA Recovered (µg)
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
Concentration of Tween 20 (%)
TE Elution
NaOH Elution
Total

Figure 23

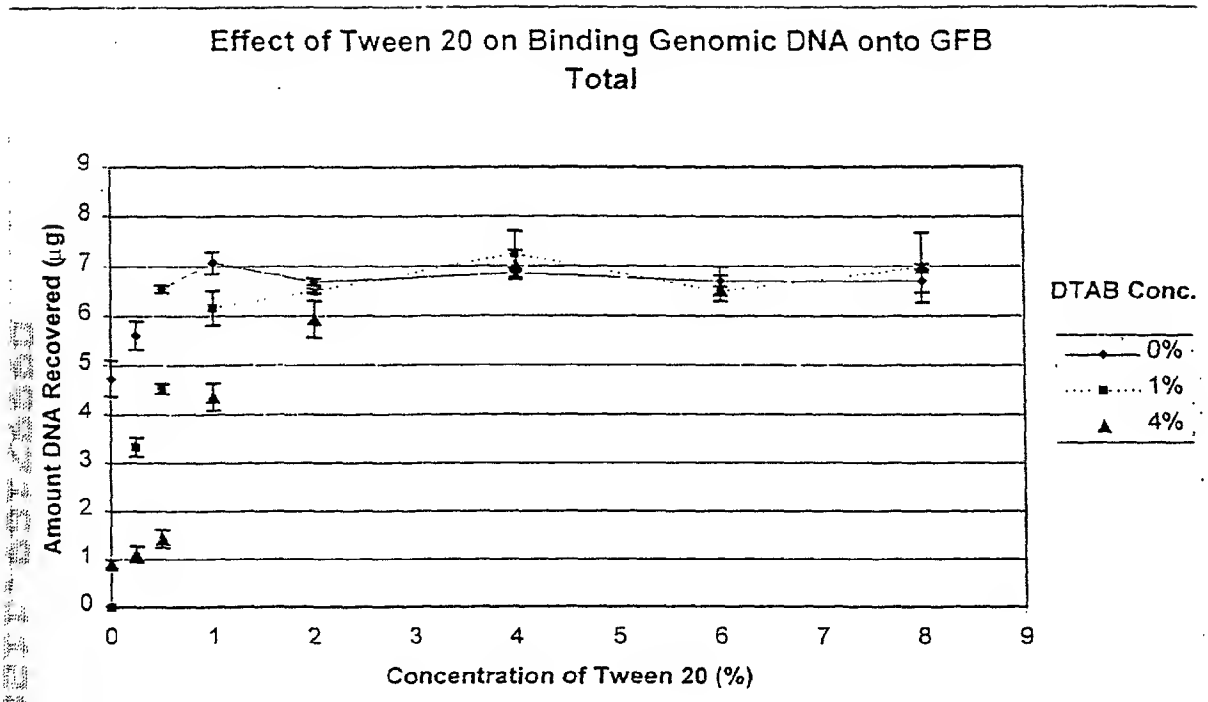

Effect of Tween 20 on Binding Genomic DNA onto GFB
Total
Amount DNA Recovered (µg)
0
1
2
3
4
5
6
7
8
9
Concentration of Tween 20 (%)
DTAB Conc.
0%
1%
4%

Figure 24

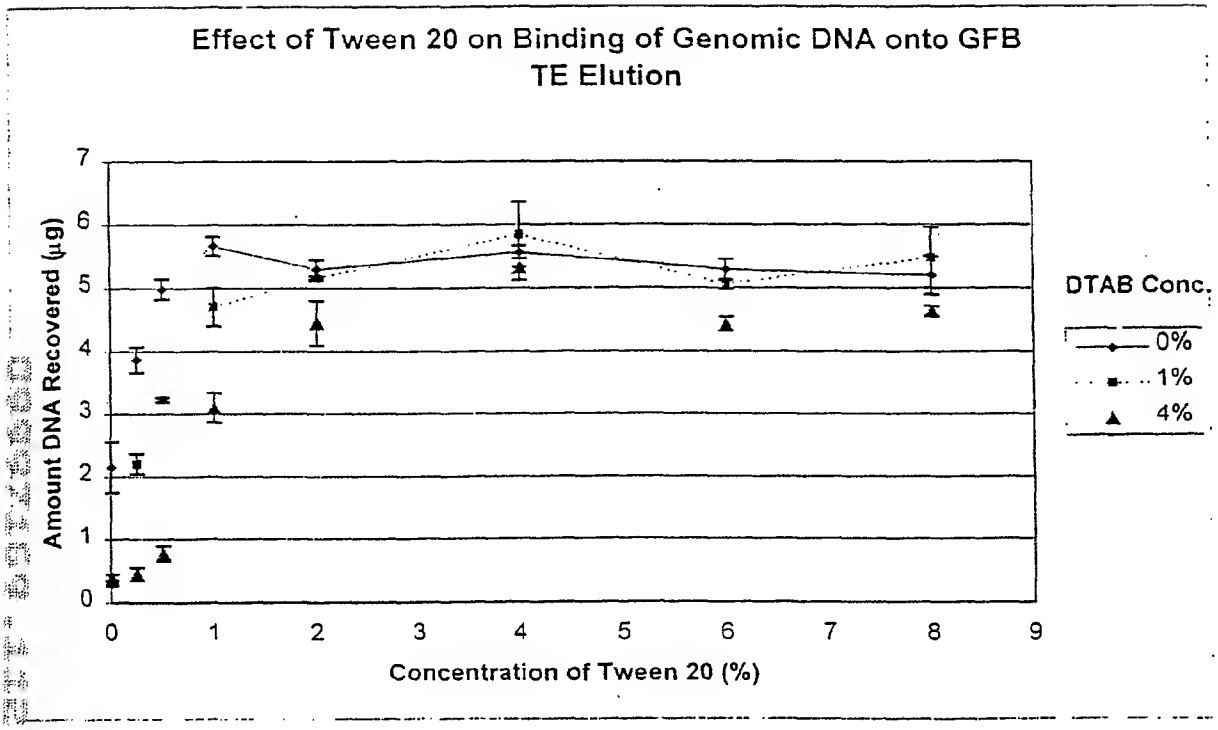

Effect of Tween 20 on Binding of Genomic DNA onto GFB
TE Elution
Amount DNA Recovered (µg)
0
1
2
3
4
5
6
7
0
1
2
3
4
5
6
7
8
9
Concentration of Tween 20 (%)
DTAB Conc.
0%
1%
4%

Figure 25

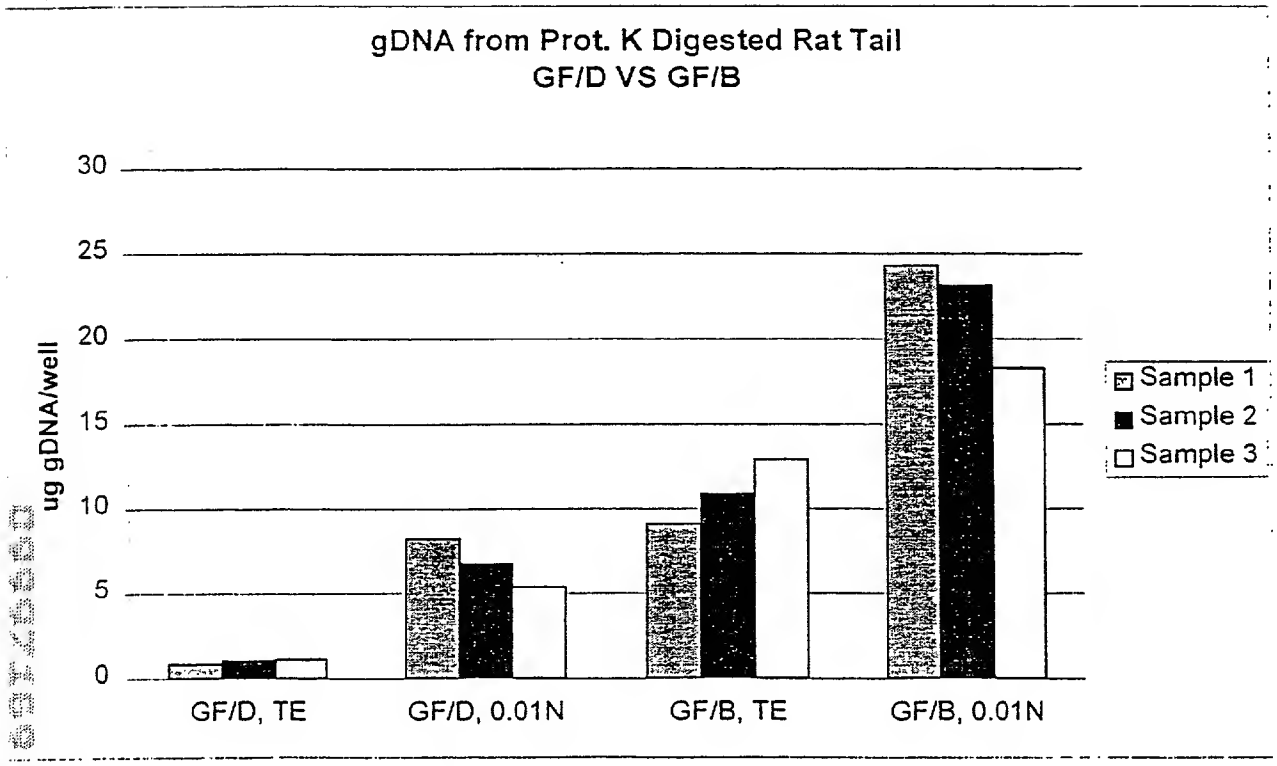

gDNA from Prot. K Digested Rat Tail
GF/D VS GF/B
ug gDNA/well
30
25
20
15
10
5
0
GF/D, TE
GF/D, 0.01N
GF/B, TE
GF/B, 0.01N
Sample 1
Sample 2
Sample 3

Figure 26

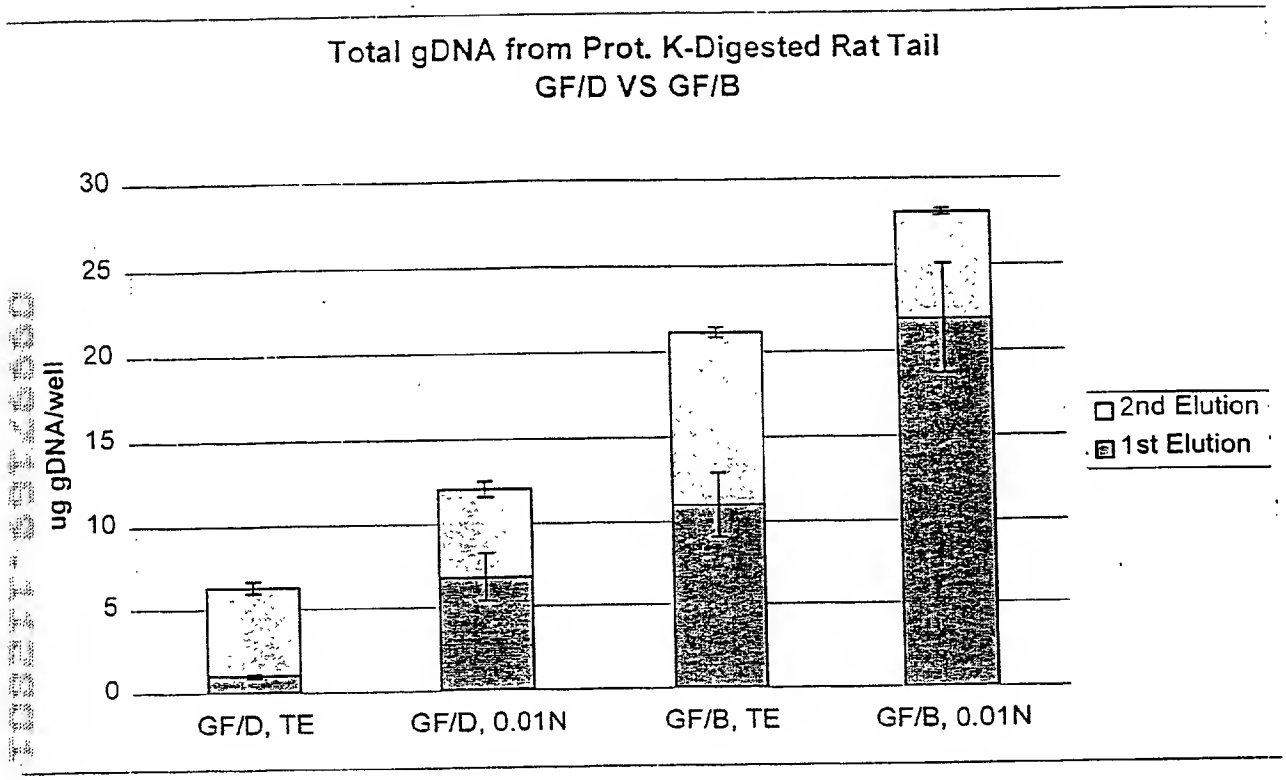
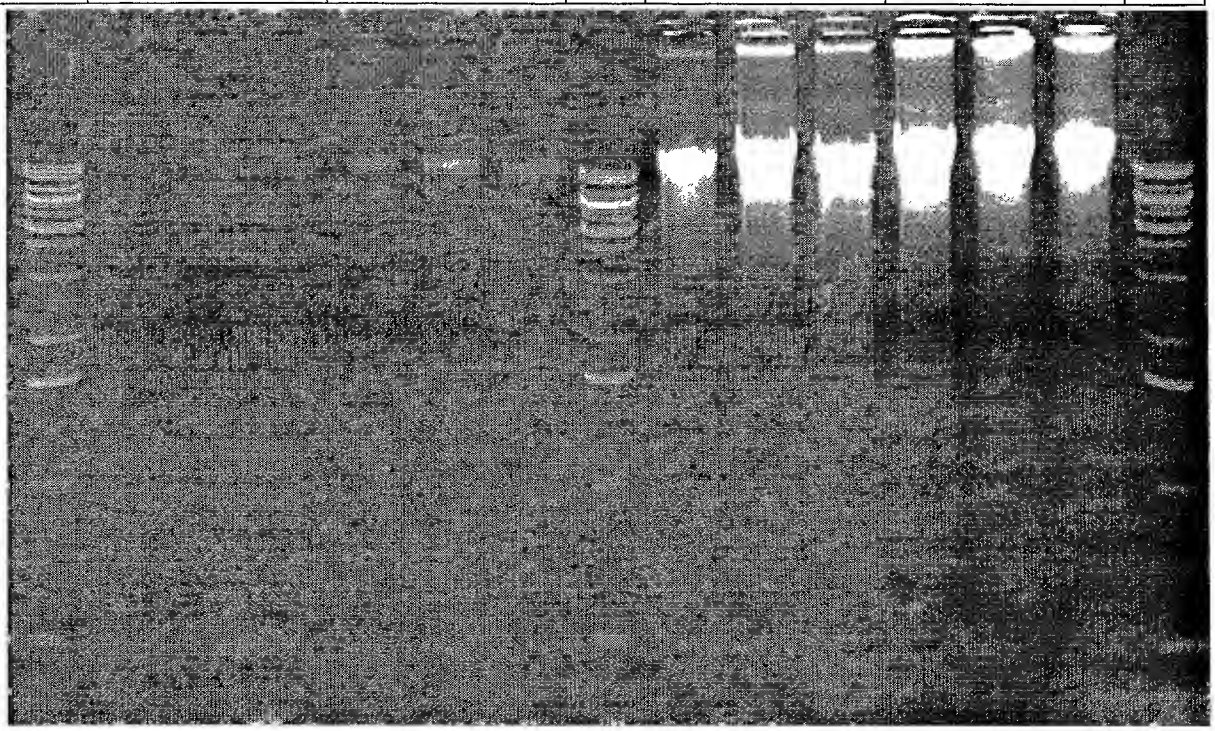

Total gDNA from Prot. K-Digested Rat Tail
GF/D VS GF/B
ug gDNA/well
30
25
20
15
10
5
0
GF/D, TE
GF/D, 0.01N
GF/B, TE
GF/B, 0.01N
2nd Elution
1st Elution

Figure 27

Membrane	GF/D			GF/B		
Elution	TE	0.1 N NaOH		TE	0.1 NaOH	



Genomic DNA from 50 mg rat tail sections digested with 1 mg of Prot. K & 1% DTAB and bound onto GF/B and GF/D membranes under 3.75 M GuSCN and 4.5 % Tween 20. The gDNA was finally eluted with of 150 mL of 1X TE and 0.01 N NaOH solutions and 20 mL was used for gel electrophoresis (1 % agarose).

Figure 28

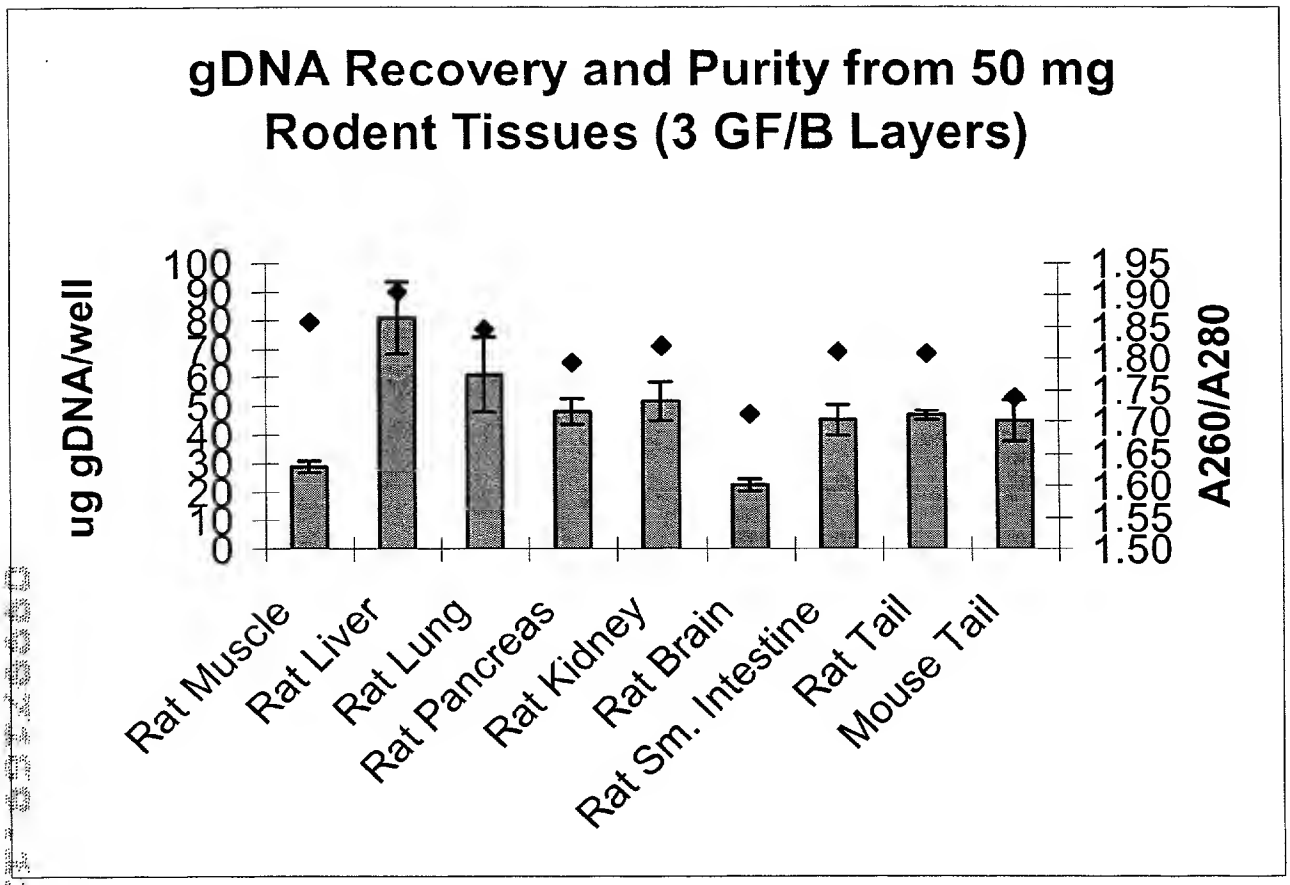
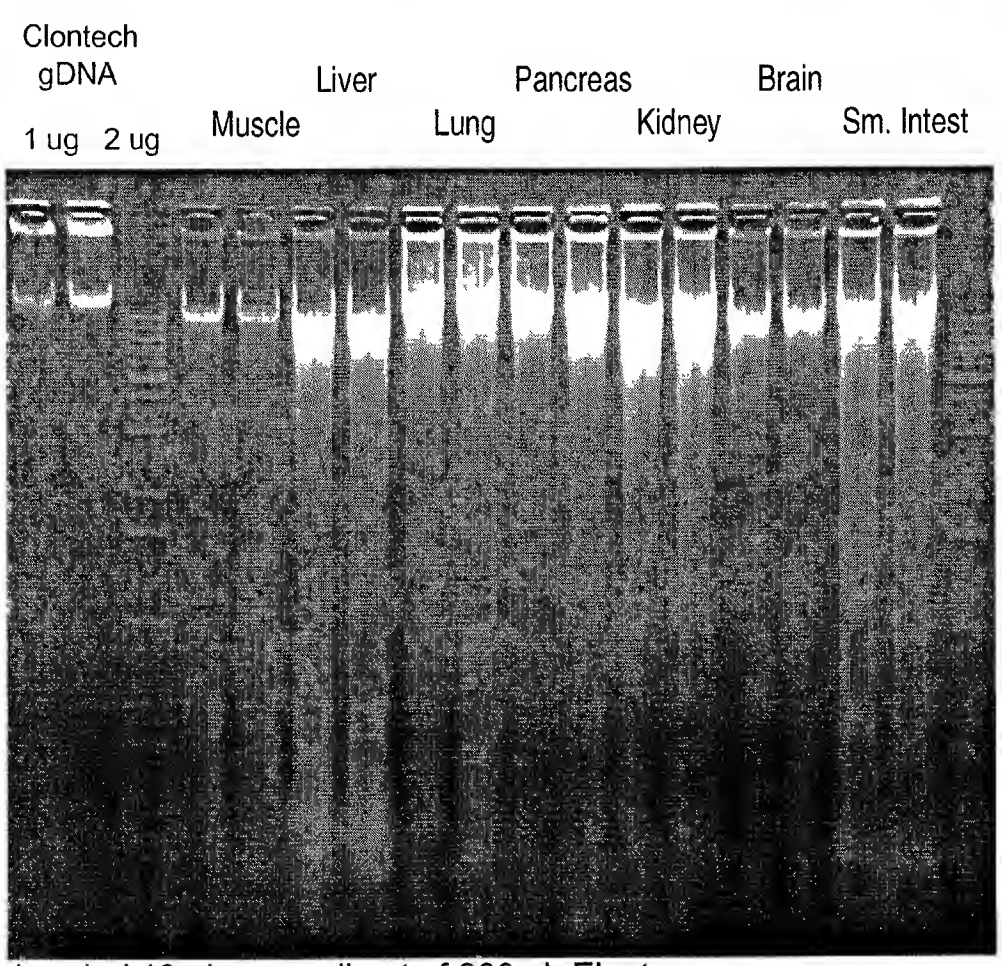

gDNA Recovery and Purity from 50 mg Rodent Tissues (3 GF/B Layers)
ug gDNA/well
A260/A280
100
90
80
70
60
50
40
30
20
10
0
1.95
1.90
1.85
1.80
1.75
1.70
1.65
1.60
1.55
1.50
Rat Muscle
Rat Liver
Rat Lung
Rat Pancreas
Rat Kidney
Rat Brain
Rat Sm. Intestine
Rat Tail
Mouse Tail

Figure 29

gDNA from 50 mg Rat Tissues



Loaded 10 uL per well out of 200 uL Eluate

gDNA fr. 50 mg Rodent Tails

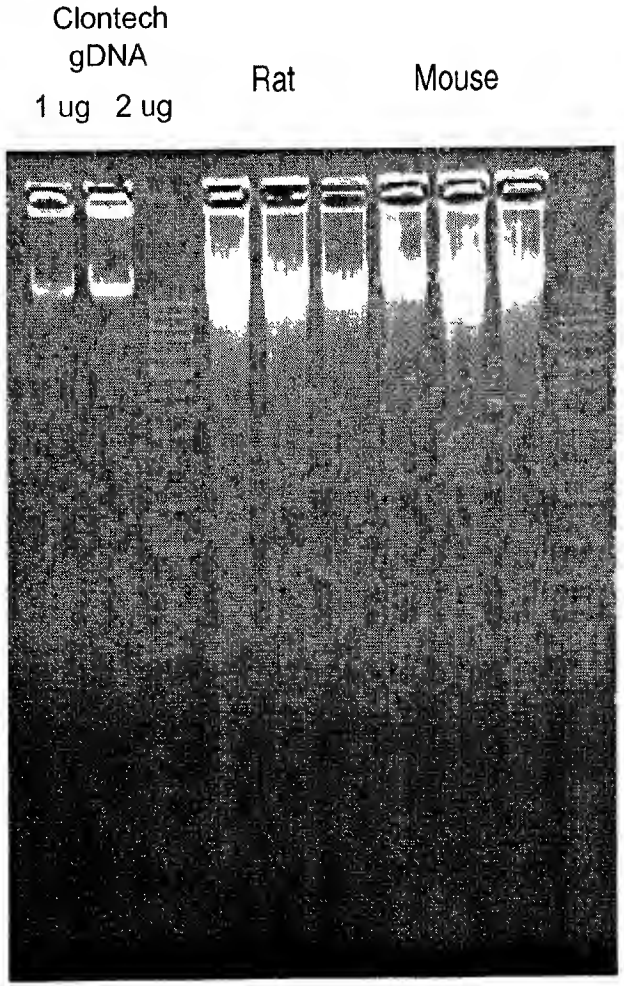


Figure 30